Influence of Molecular Oxygen on the Chlorophyll Fluorescence Decay of Green Algae

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Molecular oxygen can act as a collisional quencher of the singlet excited state of chlorophyll *a*. This effect is well described for chlorophyll *a* in various solvents but not for chlorophyll *a* in the antenna complexes of photosynthetic organisms. We studied the chlorophyll fluorescence decay of *Chlorella vulgaris cells under different oxygen concentrations but did not find any evidence for quenching by oxygen.*

Introduction

Molecular oxygen can interact with many components of the photosynthetic organism. Energy transfer or electron exchange with triplet excited molecules, like chlorophylls, leads to the formation of singlet excited molecular oxygen. This form of oxygen is highly reactive and able to oxidize lipids, pigments and proteins. The mechanisms of singlet oxygen formation and effects on photosynthetic organisms are well described (Asada and Takahashi, 1987; Telfer *et al.,* 1994).

Molecular oxygen can also act as a collisional quencher of the singlet-excited state of chlorophyll *a*. Many studies were made on chlorophyll *a* in various solvents with different concentrations of oxygen (Connolly *et al.,* 1982; Nakamura *et al.,* 1997). Collisional quenching was observed by a decreased chlorophyll fluorescence intensity and a shortening of the chlorophyll fluorescence decay time.

These observations raise the question if a similar effect occurs in photosynthetic organisms. This question is important since many studies on the photosynthetic activity are made by detecting chlorophyll fluorescence parameters that are used in subsequent calculations (Genty *et al.,* 1989; Dau, 1994).

Materials and Methods

The green alga *Chlorella vulgaris*, strain number SAG 211–11b, was obtained from the strain collection of the University of Göttingen (Schlösser, 1994). A medium recommended by Kuhl and Lorenzen (1964) was used and bubbled with clean air, containing 3% CO_2. The algae were exposed to the light of a fluorescent tube lamp for twelve hours every day. The intensity was 10 W/m^2, the temperature about 20 °C.

Measurements were made in a closed system. The algae were kept in a reservoir of about 0.2 l, where oxygen concentrations were measured with an oxygen sensor (WTW-Oxi325; Weilheim, Germany). The reservoir could be submitted to different light intensities by light emitting diodes (LED, peak wavelength of 655 nm). For the measurements presented in this paper, the algae were submitted solely to a LED light intensity of 24 W/m^2. Part of the algae were pumped to a small cuvette of about 1 ml, where the fluorescence decay measurements were performed. The algae were pumped back to the reservoir after passing the cuvette.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS II, Photosystem II; P680, primary electron donor of Photosystem II; Q_A, primary quinone in Photosystem II; τ_m, average chlorophyll fluorescence decay time; FWHM, full width at half maximum.

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Chlorophyll fluorescence decay measurements were made with a home-built setup, based on the technique of inverse time-correlated single photon counting (O'Connor and Phillips, 1984). A laser diode (Philips CQL 820 D, 655 nm) was pulsed by a fast pulse generator (AVTECH AVH-A-N; Ogdensburg, United States) with a repetition rate of 500 kHz. The generated light pulses had a typical width of 80 ps (FWHM) and an intensity lower than 3×10^8 photons per pulse and cm^2. An additional interference filter was inserted in front of the laser diode to block the minor emission in the spectral range of the detected fluorescence.

Long pass filter (Schott RG695, 1 cm thickness) were used to block the light pulses of the laser diode and to restrict the detectable chlorophyll fluorescence. Photons were detected by a fast photomultiplier (R1894, Hamamatsu); the typical time response of the measuring system was 280 ps (FWHM).

Fluorescence decay curves were analysed by iterative deconvolution techniques (Marquardt algorithm). The quality of the fits was checked by the reduced chi-square criterium χ^2_{red} and by the random distribution of the weighted residuals. All fluorescence decay curves were analysed with three exponential functions with a χ^2_{red} between 0.8 and 1.2. The obtained amplitudes α_i and decay lifetimes τ_i were used to calculate the average chlorophyll fluorescence decay time τ_m:

$$\tau_m = \frac{\int t F(t)\,dt}{\int F(t)\,dt} = \frac{\Sigma_i\, \alpha_i\, \tau_i^2}{\Sigma_i\, \alpha_i\, \tau_i} .$$

The τ_m values show a mean variation of approximately 10%.

In some measurements 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was used to block the electron transfer after the primary quinone Q_A (Velthuys, 1981). The applied concentration was 10–4 M. Fluorescence decay measurements were made after bubbling with nitrogen in order to vary the oxygen concentration of the medium. The system was closed again, the oxygen concentration was measured and the pump was switched off. Otherwise it would not be possible to obtain the state of maximal fluorescence due to the time-dependent induction of the chlorophyll fluorescence.

Results

Chlorophyll fluorescence decay measurements were performed on dark adapted algae in the presence of different oxygen concentrations in the medium. No additional illumination of the reservoir by LED light was applied. The results are shown in Table IA. Only the average chlorophyll fluorescence lifetimes are listed, because no changes of the single chlorophyll fluorescence decay parameters were observed. Since oxygen was consumed by the algae during the fluorescence decay measurements, the listed oxygen concentrations represent average values. The first chlorophyll fluorescence decay was measured in the presence of an oxygen concentration of 2.2×10^{-4} M. Oxygen was further consumed by the algae in the following time and the chlorophyll fluorescence decay was measured for different oxygen concentrations. After a measurement in the complete absence of oxygen, oxygen was bubbled into the medium resulting in a concentration of 8.1×10^{-4} M and the chlorophyll fluorescence decay was measured again. No significant effects of the oxygen concentration on the chlorophyll fluorescence decay were observed.

Table I. The average chlorophyll fluorescence decay time τ_m of dark adapted *Chlorella vulgaris* as a function of oxygen concentration; no additional illumination was applied.

A: Change of oxygen concentration due to consumption by the algae

Oxygen conc. (10^{-4}M)	2.2	1.9	1.3	0.9	0.6	0.3	0	8.1
τ_m (ps)	363	347	340	341	338	334	336	337

B: Oxygen concentration was decreased by bubbling with nitrogen

Oxygen conc. (10^{-4}M)	3.1	2.5	2.2	1.3	0.3	0	6.6
τ_m (ps)	390	414	393	408	408	409	373

Table II. The average chlorophyll fluorescence decay time τ_m of DCMU-treated dark-adapted *Chlorella vulgaris* as a function of oxygen concentration; oxygen concentration was decreased by bubbling with nitrogen.

Oxygen conc. (10^{-4}M)	2.8	0.9	0.3	0	7.2
τ_m (ps)	2407	2300	2292	2304	2268

In a second experiment, oxygen concentrations of dark adapted algae were decreased by bubbling with nitrogen. Again, no additional illumination was applied. The chlorophyll fluorescence decay measurements were performed for different oxygen concentrations (Table IB). After a measurement in the complete absence of oxygen, the oxygen concentration of the medium was increased by bubbling with molecular oxygen. Again no effects of the oxygen concentration on the chlorophyll fluorescence decay could be observed. After this experiment the algae were still able to produce oxygen, which ensured that the treatment with nitrogen did not lead to any damage of the algae.

The chlorophyll fluorescence decay of DCMU-treated dark-adapted algae was measured in the presence of different oxygen concentrations (Table II), varied by bubbling with nitrogen. A final measurement was performed after bubbling with oxygen, but again no correlation between the oxygen concentration and the chlorophyll fluorescence decay was observed.

A different situation was present when the algae were exposed to the additional LED light. A low chlorophyll fluorescence intensity (Fig. 1) and a fast average chlorophyll fluorescence decay time (data not shown) were observed before the illumination. Switching on the LEDs (24 W/m^2) lead to an increase of the chlorophyll fluorescence intensity and the typical induction process visible by the chlorophyll fluorescence. A steady state was reached after about 20 min. The oxygen concentration increased during this time, but no changes in the chlorophyll fluorescence intensity were observed (Fig. 1). After 33 min and an oxygen concentration of 6.6×10^{-4} M, the LEDs were switched off and the chlorophyll fluorescence intensity (Fig. 1) decreased with a half-time of 10 s. After the new steady state was reached the chlorophyll fluorescence intensity and average chlorophyll fluorescence decay time (data not shown) became similar to those at the beginning of the measurement. Reexposed to light after this experiment, the algae were still able to produce oxygen.

Discussion

The chlorophyll fluorescence decays of the dark adapted algae were unaffected by the oxygen concentration of the medium (Tables IA and IB). The same result was obtained for measurements on the DCMU-treated algae (Table II). No indications for oxygen acting as a collisional quencher of the excited singlet state of chlorophyll *a* were found.

A more complex situation was observed in algae submitted to light. After the LEDs were switched

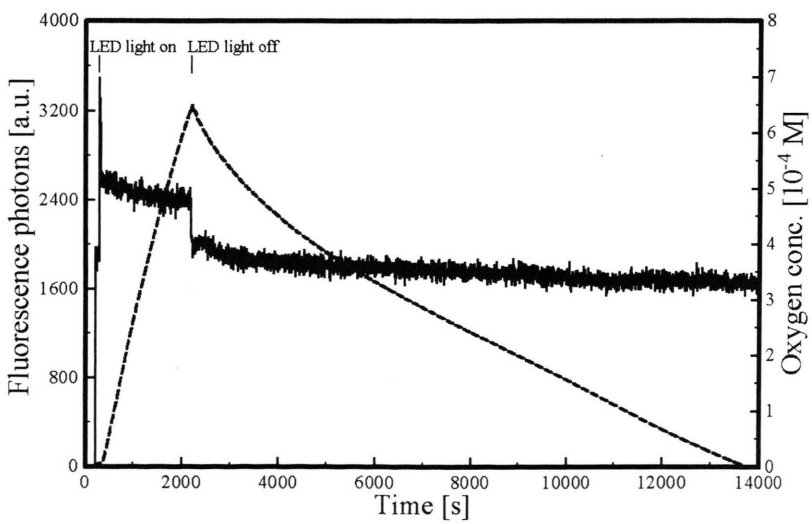

Fig. 1. Simultaneous measurement on *Chlorella vulgaris* of chlorophyll fluorescence intensity (solid line) and oxygen concentration (dashed line) under two different light conditions. 'LED light on' corresponds to a light intensity of 24 W/m^2 or a photon flux density of 130 μmol/s·m^2, respectively. The average oxygen production rate observed in the first 600 s of the 'LED light on'-period was 50 μmol/(h · mgChl). After this period the oxygen production rate decreased due to increased oxygen consumption by the algae.

on and the steady state was reached, increased chlorophyll fluorescence intensities were measured due to a reduction of primary quinones Q_A. The oxygen concentration increased in the following time but no changes in the chlorophyll fluorescence intensity were observed. Again, oxygen did not have any effects on the chlorophyll fluorescence decay of this light adapted algae, comparable to both other investigated photosynthetic states.

These results have to be compared with measurements on chlorophyll *a* solutions. Connolly *et al.* (1982) measured the chlorophyll fluorescence decay time in diethyl ether. At a chlorophyll concentration of about 3×10^{-7} M they observed a decay time of 6.1 ns in the deoxygenated solution. This decay time decreased to 5.85 ns in the aerated solution with an oxygen concentration of about 4×10^{-3} M. For increased chlorophyll concentrations of 13×10^{-6} M they observed a decay time of 7.4 ns in the deoxygenated and 6.95 ns in the aerated solution. The increase of the decay times at higher chlorophyll concentrations is caused by fluorescence reabsorption effects, but the increase of the rate of quenching by oxygen is clearly seen, too.

Similar results were obtained by Nakamura *et al.* (1997). For increased chlorophyll *a* concentrations they observed increased rate constants of quenching by oxygen, too. In additional measurements on microdroplets they observed a further increase in the rate constant of quenching by oxygen as well as a dependence on the droplet size. They ascribed this effect to the enhanced excitation energy migration at higher chlorophyll *a* concentrations accompanied with an increased propability of interaction between oxygen and the excited state. The strongest dependence was observed on a droplet of *n*-octanol with a diameter of 4 μm and a chlorophyll concentration of 3×10^{-2} M, dispersed in water. In the absence of oxygen they measured a chlorophyll fluorescence decay time of 1.45 ns that decreased to 0.48 ns in the presence of 1.65×10^{-3} M oxygen.

The highest oxygen concentration in our measurements was 26 mg/l, corresponding to a molar concentration of 8.1×10^{-4} M. The chlorophyll *a* concentration in the antenna complexes of green algae is high and should be comparable with a highly concentrated solution or a microdroplet of chlorophyll *a*. But our measurements clearly point out that no comparison can be made between the results of *in vivo* measurements on *Chlorella vulgaris* and the results on chlorophyll *a* in solution or microdroplets. Our *in vivo* measurements indicate a low oxygen concentration in the antenna complexes of the algae, despite high concentrations in the surrounding medium. Considering the results of Siefermann-Harms *et al.* (1990, 1998) it seems appropriate to expect that the large proteins embedding the antenna pigments act as a diffusional barrier for the oxygen molecules.

In conclusion, no evidence was found that dissolved molecular oxygen affects the fluorescence decay of excited singlet chlorophyll of intact *Chlorella vulgaris*. Therefore, for interpretation of fluorescence curves quenching by molecular oxygen has not to be considered.

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Asada K. and Takahashi M. (1987), Production and scavenging of active oxygen in photosynthesis. In: Photoinhibition (Kyle D. J., Osmond C. B. and Arntzen C. J., eds.). Elsevier Science Publishers, Amsterdam, pp. 227–287.

Connolly J. S., Janzen A. F. and Samuel E. B. (1982), Fluorescence lifetimes of chlorophyll *a*: Solvent, concentration and oxygen dependence. Photochem. Photobiol. **36**, 559–563.

Dau H. (1994), Molecular mechanisms and quantitative models of variable Photosystem II fluorescence. Photochem. Photobiol. **60**, 1–23.

Genty B., Briantais J. M. and Baker N. R. (1989), The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta **990**, 87–92.

Kuhl A. and Lorenzen H. (1964), Handling and culturing of *Chlorella*. In: Methods in Cell Physiology (Prescott D. M., ed.). Academic Press, New York London, Vol. **I**, pp. 152–187.

Nakamura K., Kowaki T., Scully A. D. and Hirayama S. (1997), Quenching of chlorophyll *a* fluorescence by oxygen in highly concentrated solution and microdroplets. J. Photochem. Photobiol. A **104**, 141–149.

O'Connor D. V. and Phillips D. (1984), Time-correlated single photon counting. Academic Press, London.

Schlösser U. G. (1994), SAG-Sammlung von Algenkulturen at the University of Göttingen, catalogue of strains 1994. Bot. Acta **107**, 113–186.

Siefermann-Harms D. (1990), Protective function of the apoprotein of the light-harvesting chlorophyll-*a/b*-protein complex in pigment photo-oxidation. J. Photochem. Photobiol. B **4**, 283–295.

Siefermann-Harms D. and Angerhofer A. (1998), Evidence for an O_2-barrier in the light harvesting chlorophyll-*a/b*-protein complex LHC II. Photosynth. Res. **55**, 83–94.

Telfer A., Dhami S., Bishop S. M., Phillips D. and Barber J. (1994), β-Carotene quenches singlet oxygen formed by isolated Photosystem II reaction centers. Biochemistry **33**, 14469–14474.

Velthuys B. R. (1981), Electron-dependent competition between plastoquinone and inhibitors for binding to Photosystem II. FEBS Lett. **126**, 277–281.